Box Self

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

File No. **14149-4"US" FC/ntb**

ASSISTANT COMMISSIONER FOR PATENTS,
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): **Louis-Philippe Vézine et al.**

For: **PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES**

Your petitioner prays that letters patent may be granted for the invention set forth in the enclosed specification including a disclosure, claims and declaration.

Enclosed are :

☒ Combined Declaration (unexecuted)

☐ An additional cpy of this sheet and an Assignment of the Invention to _____

☐ A certified copy of _____ on the basis of which the benefit of priority under 35 U.S.C. 119 is claimed.

☒ Declaration of small entity status.

	No. filed	Number Extra	Rate	Basic Fee $345.00
Total Claims	8		9.00	-
Multiple Dependency Fee	-	-		-
Independent Claims	1	-	39.00	-

Filing Fees	$345.00
Assignment Fee	
Total	**$345.00**

A cheque No. 008135, including the amount of $345.00 to cover the Government Assignment, Filing and Extra Claims Fee is enclosed.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Account No. 19-5113.

October 2, 2000

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Applicant or Patentee: Louis-Philippe VÉZINA et al.
Serial or Patent No.: _____ Atty. Dkt. No.: 14149-4US FC/ntb
Filed or Issued: _____
For: PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES

VERIFIED STATEMENT (DECLARATION) BY A NON-INVENTOR SUPPORTING A CLAIM BY ANOTHER FOR SMALL ENTITY STATUS

I hereby declare that I am making this verified statement to support a claim by MEDICAGO INC. for small entity status for purposes of paying reduced fees to the United States Patent and Trademark Office, regarding the invention entitled PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES by inventor(s) Louis-Philippe Vézina; and Marc-André D'Aoust described in:

(X) the specification filed herewith
( ) application serial no. _____, filed _____
( ) patent no. _____, issued _____.

I hereby declare that the said inventors qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying fees to the United States Patent and Trademark Office.

I hereby declare that the said inventors have not assigned, granted, conveyed or licensed and are under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e), except as indicated below.

Each person, concern or organization to which said inventors have assigned, granted, conveyed, or licensed or are under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

( ) no such person, concern, or organization
(X) persons, concerns or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME MEDICAGO INC.
ADDRESS 2480, rue Hochelaga, Sainte-Foy, Québec, CANADA G1K 7P4
( ) INDIVIDUAL (X) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING **France Côté**
ADDRESS OF PERSON SIGNING 102 de la Moselle, St-Lambert, Québec, Canada J4S 1W2
SIGNATURE: _____ DATE: October 2, 2000

PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to a promoter for regulating expression of foreign genes in a transgenic organism, more specifically in a leaf-specific manner in transgenic plants.

(b) Description of Prior Art

Genetic transformation of microbes have been used for more than 15 years to produce useful recombinant molecules, and applications in the pharmaceutical, cosmaceutical and dermaceutical industries are being currently exploited. This technology has expanded from microbes to plants and animals in the last ten years with the development of techniques required to adapt this general concept to complex eukaryotic organisms. Basically a gene encoding for a protein of interest or a gene encoding for an enzyme responsible for a modification of a metabolic pathway that leads to a molecule of interest, is linked in an appropriate fashion to cis-and trans-acting regulatory sequences, and transferred to a target cell where it is incorporated in the molecular machinery (in a transitory or stable fashion). The transgenic cell, or a tissue or organism regenerated from the transgenic cell will then perform transcription and translation of the transgene and therefore be enabled to accumulate the protein of interest or to perform the new metabolic reaction through the activity of the enzyme of interest.

The emerging industry of molecular farming is one of the most promising industry of the coming century. Its promise is to provide safe and renewable molecule factories for the industry. Among the applications that are currently developed are the production of low-cost monoclonal antibodies for therapeutic and diagnostic uses, the production of unlimited amounts of hormones, cytokines and other bio-active molecules for the treatment of chronicle or lethal diseases, the production of bio-safe substitutes for various blood components, the production of unlimited amounts of processing enzymes for the food and pulp industry, the production of low-cost enzymes for waste treatments, and the production of safe bio-active molecules for the cosmetic industry.

Limitations to the application of this technology has often come from the inability of transgenic organisms to accumulate adequate amounts of the recombinant product, as a result of low transcription rates, improper splicing of the messenger, instability of the foreign mRNA, poor translation rates, hyper-susceptibility of the recombinant protein to the action of endogenous proteases or hyper-susceptibility of the recombinant organism to the foreign protein which result in improper and limited growth or in the worst cases, in strong deleterious effects to the host organism. Inadequacy of production level has a direct impact on the development of applications when profit margins are narrow, or when treatment and/or disposal of residual matter causes bio-safety or environmental problems. Improvement of the accumulation level of the desired recombinant product thus appears to be one critical factor that warrants commercialization of many applications of molecular farming.

Photosynthesis is a metabolic reaction of paramount importance in the living world. It is performed by most land plants and algae, and by some bacteria. This overall reaction involves a complex assembly of electron transfer proteins spatially arranged within the thylakoid membrane system located in the chloroplast of leaf cells. This electron transport chain is coupled at one end with the photosynthetic antennae, which comprise a variety of macro-molecules, including one molecule common to all photosynthetic organism, chlorophyll, and at the other end, to the enzymes involved in NADPH and ATP synthesis, and to the Calvin cycle, involved in coupling the release of energy from NADPH and ATP with the fixation of gaseous carbon dioxide into organic molecules. Among the proteins involved in the overall photosynthetic reaction, one, Ribulose biphosphate carboxylase (Rubisco), is the most abundant protein on earth.

Photosynthesis is thus what leaf cells are dedicated to perform, and there is an obvious interest to use promoters of genes involved in such prominent tissue-specific metabolic activity when building strong leaf-specific expression cassettes for applications in plant biotechnology.

Many of the peptidic constituents of the photosynthetic apparatus are encoded by genes present in the chloroplastic genome; as an example, the heavy subunit of Rubisco, which bears the catalytic sites for CO_2 fixation, is encoded by a chloroplastic gene. However, the small

subunit of this enzyme is encoded by a nuclear gene, and thus the Rubisco holo-protein is made of subunits encoded by two different genomes. For obvious reasons, there has been a great interest in trying to use Rubisco promoters to control transcription of transgenes in leaves of transgenic plants. The promoter has been extensively characterized and its use in expression vectors is protected by United States Patent No. 4,962,028.

It would be highly desirable to be provided with a promoter for regulating expression of foreign genes in a transgenic organism, more specifically in transgenic plants.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide with a promoter for regulating expression of foreign genes in a transgenic organism, more specifically in transgenic plants.

In accordance with one embodiment of the present invention, there is provided a promoter for regulating expression of foreign genes in transgenic organisms, which comprises a promoter having the identifying characteristics of a promoter having a sequence selected from the group consisting of sequences set forth in SEQ ID NOS:1 to 3 and functional fragments or derivatives thereof, wherein said promoter is adapted to be operationally located with respect to said foreign gene for expression of said gene.

The preferred promoter of the present invention has a sequence selected from the group consisting of sequences set forth in SEQ ID NOS:1 to 3.

Preferably, the organism is a plant.

Preferably, the promoter of the present invention may be modulated by the presence or absence of light.

The prefered plant is a dicot, a monocot or a gymnosperm.

In accordance with another embodiment of the present invention, there is provided a method of regulating expression of foreign genes in transgenic organisms, comprising the steps of:

a) preparing a transgenic organism using an expression construct consisting of at least a promoter of the present invention, and an

ORF of a gene, wherein said promoter is operationally located with respect to said gene for expression of said gene.

For the purpose of the present invention the following terms are defined below.

The expression "functional fragments or derivatives thereof" is intended to mean any derivative or fragment of sequences SEQ ID NOS:1-3 which allow for an equivalent level of expression of a foreign gene as the promoter of the present invention set forth in SEQ ID NOS:1-3.

DETAILED DESCRIPTION OF THE INVENTION

Following is a detailed description of the method used to generate transgenic alfalfa lines that can be regulated in their expression of a reporter gene.

In this embodiment, a promoter having the sequence set forth in SEQ ID NOS:1-3 was then ligated to a reporter gene and a terminator, and this construct was inserted in suitable plant expression vectors for DNA bombardment onto alfalfa leaves and for *Agrobacterium* mediated DNA transfer as described by Desgagnés et al. (1995, *Plant Cell Tissue Organ Cult.* 42:129-140). These two DNA transfer methods were used to demonstrate that expression of the reporter gene can be modulated by light.

Materials and Methods

DNA sequencing

DNA sequencing was performed as described by Sanger et al (1977, *P.N.A.S. USA*, 74:5643-5647).

The resulting promoters of the present invention have the sequence as set forth in SEQ ID NOS: 1 to 3.

Construction of expression cassettes and vectors

The cassettes for expression analysis using the GUS reporter gene were assembled as follows. A promoterless GUS cassette was digested from pBI101 with HindIII and EcoRI, and was inserted into the

HindIII and EcoRI sites of the pUC19 polycloning site. The resulting plasmid was named pBI201 and was used for further constructs. Various deletion fragments of pGPlas3-2 were transcriptionally and transitionally fused at the 5'terminus of the GUS reporter gene in pBI201 and these were used for transitory expression studies using DNA bombardment. Upon identification of the adequate deletion fragment, it was subcloned into a binary plant expression vector such as pBI101 (Clonetech). These recombinant plasmids were used for stable integration through *A. tumefaciens* infection as described below.

Agrobacterium-mediated DNA transfer and regeneration of transgenic lines

The recombinant plasmids were introduced into *Agrobacterium tumefaciens* strain LBA4404 by electroporation as described in Khoudi et al (1999, *Biotechnol. Bioeng.*, 64:135-143). Selected *Agrobacterium* strains were then co-cultivated with leaf disks from genotype C5-1 for 4 days in the absence of selection pressure (kanamycin). Following this incubation period, leaf disks were washed and pampered, and then allowed to form calli onto medium B5H. Calli were then transferred for 21 days on SH medium for embryo induction and for 28 days on BOi2Y for embryo development. Torpedo-shaped embryos were removed from Boi2Y and placed on MS medium for regeneration. Kanamycin was present in all cultivation medium except for co-cultivation and regeneration on MS. This method is described in length in Desgagnés et al (1995, *Plant Cell Tissue Organ Cult.* 42:129-140). Rooted plantlets were grown to maturity in the greenhouse.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A promoter for regulating expression of foreign genes in transgenic organisms, which comprises a promoter having the identifying characteristics of a promoter having a sequence selected from the group consisting of sequences set forth in SEQ ID NOS:1 to 3 and functional fragments or derivatives thereof, wherein said promoter is adapted to be operationally located with respect to said foreign gene for expression of said gene.

2. The promoter of claim 1, wherein said promoter is modulated for transcriptional expression of said gene by presence or absence of light

3. The promoter of claim 1, wherein the promoter has a sequence selected from the group consisting of sequences set forth in SEQ ID NOS:1 to 3.

4. The promoter of claim 1, wherein said organism is a plant.

5. The promoter of claim 4, wherein said plant is a dicot, a monocot or a gymnosperm.

6. A method of regulating expression of foreign genes in transgenic organisms, comprising the steps of:

a) preparing a transgenic organism using an expression construct consisting of at least a promoter of claim 1, and an ORF of a gene, wherein said promoter is operationally located with respect to said gene for expression of said gene.

7. The method of claim 6, wherein said organism is a plant.

8. The method of claim 7, wherein said plant is a dicot, a monocot or a gymnosperm.

ABSTRACT OF THE INVENTION

The present invention relates to a promoter for regulating expression of foreign genes in transgenic organisms, which comprises a promoter having the identifying characteristics of a promoter having a sequence selected from the group consisting of sequences set forth in SEQ ID NOS:1 to 3 and functional fragments or derivatives thereof, wherein said promoter is adapted to be operationally located with respect to said foreign gene for expression of said gene.

SEQUENCE LISTING

<110> VÉZINA, Louis-Philippe
D'AOUST, Marc-André
MEDICAGO Inc.

<120> PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES

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COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63) AND POWER OF ATTORNEY	**Attorney Docket Number**	**14149-4US FC**
	First Named Inventor	**Louis-Philippe Vézina et al.**
	Complete if known	
	Application Number	
	Filing Date	
☒ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)	Group Art Unit	
	Examiner Name	

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES

the specification of which

☒ is attached hereto.

OR

☐ was filed on _____ (mm/dd/yyyy)

as United States Application Number or PCT International Application Number _____

and was amended on _____ (mm/dd/yyyy) (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/157,129	**10/04/1999**	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

COMBINED DECLARATION FOR UTILITY OR DESIGN
PATENT APPLICATION (37 CFR 1.63) AND POWER OF ATTORNEY

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

☐ Additional U.S. or PCT International application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent Trademark Office connected therewith:

☒ Customer Number: **020988**

020988
PATENT AND TRADEMARK OFFICE

Direct all correspondence to:

020988
PATENT AND TRADEMARK OFFICE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of sole or First Inventor:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Louis-Philippe	**VÉZINA**

Inventor's Signature _____ Date _____

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City **Neuville** Province or State **Québec** Postal Code Or Zip **G0A 2R0** Country **Canada**

☒ Additional inventors are being named on the supplemental Additional Inventor(s) PTO/SB/02A attached hereto.

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63) AND POWER OF ATTORNEY

PTO/SB/02A (3-97)

DECLARATION	**ADDITIONAL INVENTOR(S) Supplemental Sheet Page 1 of 1**

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) | Family Name or Surname

Marc-André | **D'AOUST**

Inventor's Signature _____ Date _____

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City **Québec** Province or State **Québec** Postal Code Or Zip **G1S 2W9** Country **Canada**

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) | Family Name or Surname

Inventor's Signature _____ Date _____

Residence:
City _____ State _____ Country _____ Citizenship _____

Post Office Address _____

City _____ Province or State _____ Postal Code Or Zip _____ Country _____

Name of Additional Joint Inventor, if any:

☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any]) | Family Name or Surname

Inventor's Signature _____ Date _____

Residence:
City _____ State _____ Country _____ Citizenship _____

Post Office Address _____

City _____ Province or State _____ Postal Code Or Zip _____ Coun try _____

☐ Additional inventors are being named on the supplemental Additional Inventor(s) PTO/SB/02A attached hereto.